Effects of Night Work on Urinary Excretion Rates of 6-Sulfatoxymelatonin, Norepinephrine and Estriol in Pregnant Women

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Abstract: It has been suggested that shift work, night work in particular, affects worker’s psychophysical health. However, the effects of night work on the health of pregnant women are not physiologically well elucidated. The effects of night work on the biological function of pregnant women were studied in the present study. Three pregnant and six non-pregnant nurses that engaged in fast-rotating shift system cooperated for the study. The 24-h urine samples were collected in two time frames, daytime (07:00 to 23:00) and nighttime (23:00 to 07:00) on the day shift, the night shift and the days off. Urinary concentrations of 6-sulfatoxymelatonin and norepinephrine were measured by enzyme-linked immunosorbent assay and estriol by radioimmunoassay. The excretion profiles of urinary 6-sulfatoxymelatonin in the pregnant and the non-pregnant women were altered on the night shift, which might relate the derangement of circadian rhythm of melatonin secretion. The urinary norepinephrine level during the night work was considerably higher in the pregnant women, which indicated the presence of higher physical burden in them due to the night work. The urinary estriol level was not significantly affected by shift, day/night and individual factors, and the effect of night work on urinary estriol level of the pregnant women remained uncertain. The results of the present study suggested that the night work of the pregnant shift workers should be much more relieved.

Key words: Shift work, Pregnant women, 6-Sulfatoxymelatonin, Norepinephrine, Estriol, Urine

Introduction

Shift work is indispensable for markedly organized work system in modern society. In Japan, it is believed that the recent revision of the Labor Standards Law will lead to an increase in the number of female night workers and that female workers tend to keep working throughout their pregnancies. It has been reported that shift work affects worker’s health. Shift work, particularly night work, influences the biological rhythms of workers, giving rise to adverse effects on their psychophysical health. Female shift workers can be more vulnerable than male workers due to their reproductive function. Since a number of adverse pregnancy outcomes, such as spontaneous abortion, premature delivery and low birth weight are found to be related to shift work, health care for the pregnant shift workers is an important social issue. However, the exact influence of shift work on the health of pregnant women has not necessarily been physiologically well elucidated. Therefore, the effects of shift work, particularly night work, on the health of pregnant workers, with special reference to biological function, need to be examined. The biological function of pregnant shift workers in the study was estimated by measuring melatonin, norepinephrine (NE) and estriol (E3).

The secretion of melatonin is under strong control of biological rhythm. Melatonin secretion is sensitive to light/dark cycle, and has circadian rhythm having a peak level at 2–4 o’clock in the morning. Serum or urinary melatonin has been used to assess the effects of work stress on biological rhythm in workers.

It is well known that catecholamines are sensitive to stimuli
from the external environment and fluctuate with stress. Norepinephrine is primarily secreted in response to physical exertion. Plasma NE concentration shows a tendency to be high during the daytime when one is in activity and low during the night when one is at rest. These characteristics of NE have lead investigators to use NE as an indicator of physical fatigue or stress\textsuperscript{10–12}.

Estriol, the major urinary estrogen, is produced in the placenta and mainly is of fetal origin. Urinary E\textsubscript{3} generally is used as an indicator of fetal well-being\textsuperscript{13, 14}.

Unlike serum levels of melatonin, NE and E\textsubscript{3} that quickly fluctuate over time, urinary metabolites provide average assessments of the levels of these hormones. Therefore, urinary excretion of 6-sulfatoxymelatonin (aMT\textsubscript{6}s), the major urinary metabolite of melatonin, NE and E\textsubscript{3} were measured in the study. It is already known that the urinary concentrations of aMT\textsubscript{6}s, NE and E\textsubscript{3} correlate well with their respective serum levels\textsuperscript{5, 15, 16}.

Here assessed was the physiological effect of shift work including night work on the health of pregnant women, with special reference to biological functions, which has not been studied in detail.

### Materials and Methods

#### Subjects

Three pregnant and six non-pregnant nurses took part in the study as the subjects (Table 1). The pregnant nurses were between 32 and 39 yr of age (average 34.3). They had heights within normal range (average 158.7 cm) and had normal body weights before gestation (average 55.0 kg). All were multiparas, and were eventless throughout their gestations. In fact, almost one month after completion of their cooperation, all pregnant subjects had a normal full term delivery. Non-pregnant subjects were all healthy, single, and manifested regular monthly cycles. They ranged in age from 22 to 38 yr (average 29.7), and had body heights and weights within normal range (average 156.8 cm, 50.8 kg). None of the nine subjects smoked, drank and were on any medication. All nine subjects were engaged in the same pattern of shift rotation system in the same hospital, and then there was no much difference in their work conditions. In order to decrease the masking effects on hormonal secretions, the nine examinees were requested to avoid excessive exercise, drinking, staying up late at night, and to keep their usual daily life style during the sampling period. Documented informed consents were obtained from all participants.

#### Shift system

All nine nurses were on a randomly-ordered fast-rotating shift system based on a backward rotation system, which was a 7 day-cycle composed of daytime, evening and night shift. Two days off were set for every five-work day. The working times for day, evening and night shift were 08:00–16:00, 16:00–00:00 and 00:00–08:00, respectively.

#### Urine collection

Taking into account the secretory profiles of hormones during a day, the 24-h urine samples were collected divided into two time frames, daytime (from 07:00 to 23:00) and nighttime (from 23:00 to 07:00). We collected urine samples on the day shift, the night shift and the days off (Table 2). On the days off and on the day shift, the daytime urine samples were collected first, followed by the nighttime samples. On the night shift, the nighttime urine samples were collected first and followed by the daytime samples. Within 24 h after collection, aliquots of them (2 ml and 5 ml) were stored in plastic cryogenic vials at –80°C until measurement for aMT\textsubscript{6}s and E\textsubscript{3}, respectively. Ten ml of urine for analysis of NE was stored at –80°C with the additive of 100 µl of 6-N hydrochloric acid\textsuperscript{5, 17}. All urine samples were collected from December 1998 to November 1999.

All pregnant subjects were examined four times: 20–23

<table>
<thead>
<tr>
<th>Table 1. Characteristics of the subjects</th>
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</thead>
<tbody>
<tr>
<td>Pregnant (n=3)</td>
</tr>
<tr>
<td>Age (yr)</td>
</tr>
<tr>
<td>34.3 (32–39)</td>
</tr>
<tr>
<td>Height (cm)</td>
</tr>
<tr>
<td>158.7 (150.0–164.0)</td>
</tr>
<tr>
<td>Weight (kg)</td>
</tr>
<tr>
<td>55.0 (49.0–62.0)</td>
</tr>
<tr>
<td>Number of children</td>
</tr>
<tr>
<td>1–2</td>
</tr>
</tbody>
</table>

Data are presented as mean (range) or range.

<table>
<thead>
<tr>
<th>Table 2. Time frames for urine sampling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daytime urine (07:00–23:00)</td>
</tr>
<tr>
<td>Days off\textsuperscript{1}</td>
</tr>
<tr>
<td>Day shift\textsuperscript{1}</td>
</tr>
<tr>
<td>Night shift\textsuperscript{2}</td>
</tr>
</tbody>
</table>

\textsuperscript{1}The daytime urine samples were collected first and followed by the nighttime samples. \textsuperscript{2}The nighttime urine samples were collected first and followed by the daytime samples.
wk of gestation (G1), 26–28 wk (G2), 32 wk (G3) and 36 wk (G4) (Table 3). They were exempted from night duty after 32 wk and took maternity leave after 36 wk of gestation. Therefore, their urine samples were collected and examined on the days off and on the day shift for the G3 and only on the days off for the G4. All non-pregnant subjects were examined once per each work shift during the follicular phase of their monthly cycle.

Measurement

Urinary concentrations (ng/ml) of aMT6s and free NE were measured in duplicates by enzyme-linked immunosorbent assay (ELISA, IBL, Hamburg, Germany) and E3 by radioimmunoassay (RIA, SRL, Tokyo, Japan). For comparison of hormone levels among the three shifts the results were standardized as an excretion rates per hour (µg/h). The correction of these levels by urinary creatinine concentration gave the same values as the standardized ones. Thus, the results were presented as the excretion rates.

Considering the sensitivity of melatonin secretion to light/dark cycle, illumination intensities at the work places and at homes were measured for all subjects by a lux meter (ANA-F9, Tokyo-Koden, Tokyo, Japan). In addition, sleep or nap times during each shift were recorded by each examinee (Table 4).

Statistical analysis

Statistical analysis was performed by analysis of variance (ANOVA).

Results

I. Urinary aMT6s and NE excretion rates of pregnant and non-pregnant subjects

The effects of shift work on urinary excretion rates of aMT6s and free NE were compared between the pregnant and the non-pregnant women. Statistical analysis was performed by 4-way ANOVA. For this analysis, the data from the collection time frames of G1 through G2 for the pregnant and the non-pregnant subjects were used. Data included measurements at all work shifts; the days off, the day shift and the night shift (Table 3, Analysis A).

aMT6s:

The day and the night aMT6s levels (µg/h) of the pregnant and the non-pregnant subjects on the three work shifts were given in Fig. 1. Table 5 shows the results revealed by 4-way ANOVA for aMT6s level, including the 2-way interactions, in relation to the four factors: shift, day/night, pregnancy and individual.

The day levels of aMT6s were remarkably lower than the night levels on all shifts for both the pregnant and the non-pregnant subjects. For the day levels of aMT6s, there was no remarkable difference among the three shifts for both the pregnant and the non-pregnant subjects.

For the night levels of aMT6s, the night shift revealed evidently lower levels than the days off or the day shift for both the pregnant and the non-pregnant subjects. The aMT6s night levels of the pregnant subjects tended to be higher than those of the non-pregnant subjects on all corresponding shifts. Particularly on the night shift, the pregnant subjects’ night level was considerably higher than that of the non-pregnant subjects (2.30 µg/h and 1.42 µg/h, respectively) (Fig. 1).

The results by the ANOVA showed significant effects of the shift, day/night and individual factors on the aMT6s level (P<0.01, P<0.001 and P<0.001, respectively), with an exception for the pregnancy factor. The interactions between the shift and the day/night factors, and between the day/night and the individual factors were also significant (P<0.01 and P<0.001, respectively) (Table 5).

NE:

The day and the night urinary NE levels (µg/h) of the

Table 3. Urine sampling for pregnant subjects with gestational stages and for non-pregnant ones

<table>
<thead>
<tr>
<th></th>
<th>Pregnant (n=3)</th>
<th>Non-pregnant (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G1</td>
<td>G2</td>
</tr>
<tr>
<td>Days off</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>Day shift</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>Night shift</td>
<td>O</td>
<td>O</td>
</tr>
</tbody>
</table>

O: sampling, ×: no sampling.
Sampling in the boxes with solid-line are for Analysis A, and those in the box with dashed-line for Analysis B.

Table 4. Hours of sleep/nap on work shift

<table>
<thead>
<tr>
<th></th>
<th>Pregnant (n=3)</th>
<th>Non-pregnant (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days off</td>
<td>sleep 6.3 ± 0.48</td>
<td>6.9 ± 0.08</td>
</tr>
<tr>
<td>Day shift</td>
<td>sleep 6.0 ± 0.48</td>
<td>7.2 ± 0.28</td>
</tr>
<tr>
<td>Night shift</td>
<td>nap 1.0 ± 0.39</td>
<td>1.0 ± 0.63</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SE.
EFFECTS OF NIGHT WORK ON PREGNANT WOMEN

On the night shift, however, the night levels of urinary NE were elevated, and slightly higher than the day levels both for the pregnant and the non-pregnant subjects. For the day levels of urinary NE in the non-pregnant subjects, the day shift showed the highest level and the night shift showed the lowest (1.86 µg/h and 1.25 µg/h, respectively). Whereas, for the pregnant subjects, the night shift showed the highest level, while the day shift showed the lowest (2.77 µg/h and 2.40 µg/h, respectively). For the night levels of urinary NE, the night shift showed the highest for both the pregnant and the non-pregnant subjects (2.82 µg/h and 1.35 µg/h, respectively). Both the day and the night urinary NE levels of the pregnant shift workers were higher than those of the non-pregnant workers on all the corresponding shifts. Particularly on the night shift, the pregnant subjects’ data were more than twice higher than those of the non-pregnant both for the daytime and the nighttime levels (Fig. 2).

The results by the ANOVA showed significant effects of all factors: shift, day/night, pregnancy and individual (P<0.05, P<0.001, P<0.05 and P<0.001, respectively). The interaction between the shift and the day/night factor was also statistically significant (P<0.01) (Table 5).

II. Urinary E3 excretion rates of pregnant subjects

The day and the night E3 levels (µg/h) of the pregnant subjects on the three work shifts during the G1 and the G2 gestational stages were demonstrated in Fig. 3. Table 6 shows the results revealed by 3-way ANOVA for urinary E3 level, including the 2-way interactions, in relation to the three factors: shift, day/night and individual.

Both daytime and nighttime levels of urinary E3 revealed high values on the night shift (182.7 µg/h and 174.8 µg/h, respectively) and low on the days off (146.5 µg/h and 154.4 µg/h, respectively).
Both on the days off and the day shift, the daytime levels were slightly lower than the nighttime levels. On the night shift, the nighttime level was slightly lower than the daytime level (Fig. 3).

The results by 3-way ANOVA for urinary E$_3$ levels of the pregnant subjects showed no significant effects of the three factors (Table 6).

### III. The whole day values of urinary aMT6s, NE and E$_3$ with gestational stages

The long-term changes of whole day values (mean per hour of 24-h urine value: µg/h) in urinary aMT6s, NE and E$_3$ levels on the days off from the G1 to the G4 were depicted in Fig. 4. The whole day values of urinary aMT6s, NE and E$_3$ of pregnant subjects were analyzed using 2-way ANOVA in relation to the two factors: gestational stage and individual (Table 3. Analysis B).

Urinary aMT6s levels of the pregnant subjects showed no considerable change from the G1 to the G4 (Fig. 4). Furthermore, urinary aMT6s levels of the pregnant subjects didn’t appear to be different from that of the non-pregnant individuals. Average urinary NE levels of the pregnant subjects appeared to be steadily decreased from the G1 to the G4. However, no statistical significance was found. The average urinary NE levels of the pregnant subjects were higher than that of the non-pregnant subjects, even at the G4 where the value was the lowest. Urinary E$_3$ levels of the pregnant subjects were significantly increased from the G1 to the G4 (P<0.01).

The results by 2-way ANOVA for the data of the pregnant subjects showed significant effects of individual factor both on the aMT6s and NE levels.

### Table 6. The results of 3-way ANOVA for urinary E$_3$

<table>
<thead>
<tr>
<th>Factor</th>
<th>E3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shift (A)</td>
<td>0.90</td>
</tr>
<tr>
<td>Day/night (B)</td>
<td>0.01</td>
</tr>
<tr>
<td>Individual (C)</td>
<td>3.03</td>
</tr>
<tr>
<td>A × B</td>
<td>0.08</td>
</tr>
<tr>
<td>A × C</td>
<td>0.98</td>
</tr>
<tr>
<td>B × C</td>
<td>1.01</td>
</tr>
</tbody>
</table>

The upper and lower rows of each factor represent F and P values, respectively. The number of pregnant subjects was three, and corresponding data from the G1 and the G2. The number of non-pregnant subjects was six.
Among all pregnant individuals who cooperated in this study, only three took uneventful course up to delivery and remained as available subjects for the analysis. Others were excluded from the analysis because of adverse outcomes such as threatened abortion or premature delivery. Urine samples of the three pregnant subjects were collected from the G1 to the G4, and urinary aMT6s, NE and E3 were measured. Thus, the effects of shift work on urinary excretion rates of these hormones with gestational age were studied. The data of the pregnant subjects were also compared with those of the non-pregnant subjects.

Hospital nurses engaged in shift work tend to have irregular menstruation. Among all non-pregnant nurses who agreed to cooperate with this study, only six had regular menstrual cycles and could participate in the study as subjects. The association of melatonin secretion with menstrual cycle is on controversy. In order to avoid this problem, urine samples from the non-pregnant subjects were collected exclusively at their follicular phase. It took a long period to complete all samplings, as it was needed to wait for the appropriate timing to match that of the three work shifts with their follicular phase.

Twenty-four hour urine was collected dividing into two timeframes, sixteen hours during daytime and eight hours at night, and the results were satisfactory.

aMT6s

Both on the days off and the day shift, urinary aMT6s levels at night were significantly higher than those at daytime, which was observed in both the pregnant and the non-pregnant subjects. These results are consistent with the physiological circadian rhythm of melatonin secretion. On the night shift, the urinary aMT6s levels during night work were decreased, and its day/night difference became small. The melatonin secretion during night work is variable in literatures, i.e., unaltered or decreased. The results by ANOVA in the present study evidently indicated that the urinary excretion rates of aMT6s during daytime and nighttime were affected by work shifts, and supported the latter.

Since the present experiment was conducted on two fractions of 24-h urine, during daytime and nighttime, it might not demonstrate the detailed circadian rhythm of melatonin secretion, but it surely exhibited that the day/night excretory profile of urinary aMT6s was altered.

The decrease in the level of aMT6s during night work might be affected by artificial illumination and sleep deprivation. Melatonin secretion is sensitive to light/dark cycle, and its secretion is suppressed at 500–800 lux. This suppression is reflected in the urinary aMT6s level. The present experiment was carried out at 500–800 lux in the work places and at 250–300 lux in all homes. Thus, the decrease in urinary aMT6s level during night work in the present study might partially be the affect by the light suppression.

The effect of sleep/wake state on melatonin secretion is not established. Some reports suggest that the sleep/wake pattern affects melatonin secretion, while others suggest it does not. The nap during the night work in this study was an hour on average both in the pregnant and the non-pregnant subjects. Thus, the decrease in urinary aMT6s level during the night work observed in this study might be associated with the shortage of sleep.
The change of excretory profile of aMT6s in the night shift might reflect the derangement of the circadian rhythm of melatonin secretion, and such deranged rhythm would cause an increased physical burden of the night workers. Although there was no statistical significance, the aMT6s levels of the pregnant subjects were apt to be higher than those of the non-pregnant. The same tendency was reported in other articles. However, the association of melatonin secretion with reproductive function such as pregnancy remains unestablished.

The change in melatonin level with advancing gestational age is also controversial. Some researchers have reported that melatonin levels might be altered during gestation, while others reported unaltered. As for this study on urinary metabolites, aMT6s, it displayed no significant alterations from the G1 when the subjects were on work to the G4 when out of work. Thus, on the long term observation, the urinary aMT6s level of the pregnant subjects was unlikely to be affected by work only. Here, the data of pregnant shift workers are not compared with those of corresponding pregnant women unemployed. Further study in this respect would be necessary.

**NE**

Urinary NE levels both on the days off and the day shift revealed evident difference between daytime and nighttime, i.e., high in the daytime and low in the nighttime. This was observed in both the pregnant and the non-pregnant subjects. These results do not contradict the physiological profile of normal NE secretion. Urinary NE levels on the night work, however, were significantly increased in both the pregnant and the non-pregnant subjects, and the evident day/night differences mentioned above were disappeared. Thus, the urinary NE excretion rates of nighttime were significantly affected by the work shifts in both the pregnant and the non-pregnant subjects. Night work generally raises the NE level to flatten the physiological rhythm of NE secretion. The results by ANOVA in this study indicated that this applies not only to non-pregnant but also to pregnant subjects. The enhanced NE levels during night work suggest the increase in physical burden to both the non-pregnant and the pregnant individuals.

It is reported that urinary and plasma NE level is not different between pregnant and non-pregnant individuals. However, the results of this study implied that urinary NE levels of the pregnant subjects were significantly higher than those of the non-pregnant subjects. In particular, the urinary NE levels during night work of the pregnant subjects displayed twice as high as that of the non-pregnant individuals. These findings indicated that the physical burden by the night work would be larger in the pregnant subjects than in the non-pregnant. Besides, the urinary NE level during daytime immediately after night work was also more than two times higher in the pregnant subjects than in the non-pregnant subjects. In the non-pregnant subjects, the daytime NE level immediately after the night work was the lowest among the daytime levels of the three shifts, while it was highest in the pregnant subjects. These findings suggested that pregnancy shift workers would take insufficient rest after night work, presumably carrying fatigue from the night work over the next day.

The whole-day values of urinary NE excretion from the G1, working period, to the G4, non-working period, were consistently declined. The NE levels in serum or in urine during pregnancy are on controversy such as increased, decreased or not changed. None of these reports, however, referred to the relationship between NE level and workload of pregnant shift workers. The decrease in the urinary NE level with advancing pregnancy in this study might reflect relief of workload by such as exemption from the night work and taking the maternity leave. However, urinary NE levels of the pregnant subjects were higher than that of the non-pregnant subjects even at the G4 when the workload was completely exempted. All of the pregnant participants in this study were caring for their children during the sampling period. The high urinary NE level might reflect not only the workload but also burden from household duties and child-rearing.

In spite of higher NE level of the pregnant subjects than that of the non-pregnant, the former stayed healthy and gave birth to newborns uneventfully. It might be said, therefore, that the shift work including night work, indeed was not as much as to affect maternal and fetal health, but it surely affected the urinary NE excretion rates. It is reported that, elevated NE level produces uterine contraction, rendering deleterious effects on the health of the mother and fetus. These reports as well as the present study suggested that some policy to relieve the workload of the pregnant night workers should be executed.

**E3**

The effect of shift work on urinary E3 excretion rates of pregnant individuals is rarely reported. The present study displayed that urinary E3 level of pregnant subjects during the daytime was lower than that during the nighttime on the days off and on the day shift. The circadian rhythm of urinary E3 is on controversy. Wolfrum reported that E3 level has no clear circadian rhythm. Others reported that E3 level is
low during daytime and high at night\textsuperscript{77}. The results of this study are similar to the latter.

On the night shift, urinary E\textsubscript{3} level during the daytime was slightly higher than that during the nighttime. The effect of physical activity on E\textsubscript{3} level of pregnant women is controversial. Some reported that E\textsubscript{3} level after exercise was increased and other reported it was decreased after an increase\textsuperscript{38,39}. In this study, urinary E\textsubscript{3} level of the pregnant subjects seems to be lower during physical activity or work rather than at rest. When this issue is viewed from advancing pregnancy, the G1, working period, showed significantly lower levels than the G4, non-working period. The low urinary E\textsubscript{3} level on working period casts a concern that labor might affect adversely on fetal health. However, the increase of urinary E\textsubscript{3} level toward late pregnancy is consistent with a physiological change that is seen in normal development of fetus\textsuperscript{13,14}. As stated above, all of the pregnant participants in this study gave birth to healthy neonates a month after their final cooperation. This physiological increase of urinary E\textsubscript{3} level with advancing pregnancy makes this situation ambiguous. According to our results revealed by 3-way ANOVA, shift work was not a significant factor influencing urinary E\textsubscript{3} excretion rates in the pregnant subjects. Thus, this study could not establish clear effect of the shift work on urinary E\textsubscript{3} excretion rates in the pregnant subjects.

\textit{Future tasks}

Since the study described here is an experimental study with combination of field visits, the control for masking effects on hormone secretion might be incomplete. This should be taken into consideration in designing future study to control possible masking effects on hormone secretion. The individual factor was statistically significant for both levels of urinary aMT\textsubscript{6}s and NE. Increasing the number of subject is an issue in the future studies. Furthermore, in order to elucidate the effect of night work on the health of pregnant individuals, data of pregnant persons who are in work needs to be compared with those of pregnant persons who are not in work.

\textbf{Acknowledgments}

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\textbf{References}

13) Shimizu Y, Yanaihara T (1999) Estrogen: estron (E\textsubscript{1}), estradiol (E\textsubscript{2}), estriol (E\textsubscript{3}) and estetrol (E\textsubscript{4}). Nippon Rinsho \textbf{57}, 252–65 (in Japanese).


